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14. ABSTRACT
This project is exploring the hypothesis that higher intake of dietary heterocyclic amines is associated with elevated risk of prostate cancer. It is also exploring the potential that polymorphic variation in key metabolism genes may affect risk. The project is using epidemiological methods to conduct a case-control study. We successful completed interviews on 387 cases and 343 controls. Biosamples were obtained on over 96% of participants. The questionnaires have been computerized and initial genotyping results have been obtained. Data are being prepared for final data analysis.

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INTRODUCTION

The etiology of prostate cancer is currently unclear. There is evidence to support a link to variation in androgen metabolism. However, the role of other environmental factors is controversial. Epidemiological studies have shown an increased risk in men who eat large amounts of meat. We hypothesize that this may be related to the method of cooking the meat: cooking meat at high temperatures produces heterocyclic amines, exposure to which has been shown to cause prostate cancer in rodent models. Since these chemicals require metabolic activation to become carcinogenic, we further hypothesize that variation in key metabolic enzymes will modify the risk associated with dietary intake. This study proposed to conduct a case-control study of 400 men with prostate cancer and 400 men without prostate cancer. All men will complete a self-administered diet questionnaire and a one hour interview; they will provide a blood or saliva sample for DNA extraction. Laboratory analyses will be conducted to genotype all subjects on a range of about 100 polymorphic variants. As of the date of this report, we have completed all field work, recruiting slightly below our target (387 cases and 343 controls). Biosamples were obtained from over 96% of participants.

BODY

This report reflects progress on the study from initial funding in January 2005 to the end of a one year no-cost extension. All tasks based on the original Statement of Work should have been completed by now. Tasks 1 and 2 have been completed in full. As noted in an earlier report, Task 3 was dropped due to the refusal of our local IRB to provide approval. Task 4 was deferred until after completion of field work due to an unfavorable exchange rate between the Canadian and US dollars which adversely affected the budget. A request was made to obtain supplemental funding to perform this tasks but this request was denied since the review committee felt that this task was not an essential component of the project. As a result, Task 4 should now be removed from the SOW. Tasks 5 and 6 have largely been completed. However, as noted elsewhere, we are still working to complete the final genotyping and data editing. As a result of the delay in completing tasks 5 and 6, tasks 7 and 8 are still pending.

As noted in earlier reports, the project encountered serious and disruptive personnel issues in the first nine months of operation. This crisis essentially froze progress on the project for over two months. The ultimate resolution of this crisis was the departure from the project of two of the three project staff. These staff members were replaced with new employees who began work in January, 2006. At that time, it was necessary to re-design many of the administrative protocols and re-develop many of the field work methods. This delayed obtaining final IRB approval, and, unfortunately, led to a significant delay in progress towards the final targets.

Essentially, as a result of these problems, the project was delayed by about 7-8 months. Identification and interviewing of all subjects was to have been completed by month 19 (September, 2006). In reality, the final candidate case identification review was conducted in

December, 2006 with the final interviews being completed in May, 2007. The final control subject interviews were all completed by early June, 2007. While we came close to our recruitment targets, the small short-fall in recruitment (primarily in control subjects) was largely due to the budgetary implications of the delay in the project.

In addition to subjects enrolled into the main case group, we were able to supplement the study by enrolling a sub-set of otherwise ineligible men into a sub-study. The case identification method required by our local IRB meant that we are unable to establish final subject eligibility until after subjects had agreed to participate in the study. We found that about 15-20% of subjects who volunteered for the project lived outside our target region or had been diagnosed prior to our date of eligibility. Since these men are keen to participate in this research, they were mailed a version of our main study questionnaire for self-completion, and a saliva kit from which DNA was extracted. Overall, we have mailed sub-study packages to 94 men of whom 74 have returned them. The information from this group will be used to supplement the main case group where we are convinced that no bias will be introduced.

Task #1: Develop study protocols, questionnaires, recruitment procedures, etc. (Months 1-6)

This task has been fully completed.

The final IRB approval of the study questionnaires (in translated form) took place around June, 2006.

a) *The study coordinator will be hired (Month 1). (S)he will hire the interviewers.* The study coordinator was hired April 1, 2005. Unfortunately, she had to be replaced in December, 2005. In January, 2006, the project hired two research officers with responsibility to running the field work aspects of the study. These staff members have performed admirably.

b) *The questionnaires will be finalized and translated into French;* The questionnaires were finalized in November, 2005. However, we could not finalize the French translation until after IRB approval had been received for the final English version (in March 2006). The final French versions of the questionnaires were approved in June, 2006.

c) *The recruitment strategy will be tested in Month 4 when we will use the system to identify subjects for a pilot study.* The pilot study took place on schedule. The pilot study for the cases involved men diagnosed in June/July, 2005 at the Ottawa Hospital who were mainly interviewed in August and September. The case recruitment protocol worked successfully. This protocol was extended to the second study site (the Queensway-Carleton Hospital) in May, 2006. The control pilot study identified problems with the proposed protocol, leading to further pilot work (see a later item).

d) *The interviewers will be trained in the interview procedures. In Month 4, we will complete a pilot study using study procedures, including blood collection.* The initial interviewer training took place in August, 2005. The initial plan for blood collection (using the interviewers) had to be modified since we were unable to recruit interviewers who were legally qualified to collect blood samples. The revised blood collection protocol (based on a dedicated laboratory technologist) was tested in September, 2005 and worked well. Additional field staff were hired in July, 2006 and the interviewer training was repeated at that time. The additional staff were required to replace field work staff who had departed and to accommodate the higher than anticipated monthly interview load necessitated by the delay in starting the main field work.

e) *The control selection methods will have been developed, including the sampling frame and a schedule for mailings to meet recruitment and matching targets.* Control recruitment was also tested in summer, 2005 but the proposed strategy was not successful (imposing very high work load requirements to recruit subjects). A modified version was tested in October but results were also not acceptable. A third version was tested in spring, 2006 (delayed due to the personnel issue mentioned above) and has proven to be satisfactory. The new protocol (based on a modified random digit dialing) was successfully implemented.

f) *All field work procedures will be finalized and operational by the end of Month 5.* All case recruitment procedures were finalized by the end of August, 2005 (month 5). Control recruitment procedures were not finalized until spring, 2006. Administrative procedures in the research office required major revision in January, 2006 but were finalized by the end of February 2006.

g) *The primary study data base will be prepared and ready for use by the end of Month 6.* A version of the primary data base was developed by the end of 2005. It has undergone two major revisions since then. All of the study databases are now fully operational.

Task #2: Recruit 400 men with prostate cancer and 400 men without prostate cancer. Complete interviews on all of the subjects (Months 5-19).

This task has been completed.

A detailed report on the subject recruitment activities is attached to this final report. In summary, the study identified 976 candidate case subjects, 84 in the pilot phase and 892 in the main study phase. Attending physicians mailed requests for permission to release their patient's name to the study to 756 subjects (49 in the pilot phase and 707 in the main study). Permission to release their name to the research study was obtained from 32 pilot phase subjects and 513 main study subjects. Once the name release was obtained, we were able to determine full eligibility: 23 pilot phase subjects and 407 main study subjects met those criteria. All of these subjects (and 2 main study participants who 'slipped through' the eligibility screen during the first months of field work) were scheduled for an interview. Questionnaires were obtained on 414 subjects (22 in the pilot phase and 392 in the main study). Biosamples has been obtained

from 408 subjects (22 in the pilot phase and 386 in the main study) with most samples (94%) being blood.

The study identified 419 candidate control subjects, 32 in the pilot phase and 387 in the main study phase. In the main study, 12 candidate controls were ineligible due to prior prostate cancers or other issues. A further 23 subjects changed their mind and declined to complete the interview and nine subjects could not be contacted to schedule an interview. Overall, we obtained interviews in 343 control subjects in the main study (92% of eligible subjects). Questionnaires were been returned from all but one control subject. Biosamples were obtained from 354 subjects (21 in the pilot phase and 333 in the main study) with most samples (91%) being blood.

In addition, 94 subjects were invited to participate in the sub-study. Completed questionnaires and saliva samples were obtained from 74 subjects.

a) *Case recruitment will be implemented and monitored to ensure we are identifying at least 35 new cases per month.* The start of active case recruitment was delayed until April, 2006 due to the major staffing issues mentioned above, complicated by delays in obtaining final IRB clearance. As noted, the case recruitment protocol recruited men with an initial diagnosis of prostate cancer between August, 2005 and December, 2006. During the 17 months of case identification, we identified an average of 44 candidate cases per month. After allowing for ineligible cases and men who declined to participate, the sustained interview rate was about 31 cases per month for the period of active field work (April, 2006 to May, 2007).

b) *The control recruitment strategy will be implemented with a recruitment rate of 35 cases per month.* The final control recruitment protocol was established in July, 2006 following three pilot evaluations of alternative strategies. Staff to implement the protocol were hired and trained during July and August, 2006 with the protocol begin implemented in full starting in September, 2006. Between that date and the end of field work in May, 2007, we successfully interviewed 343 controls in the main study for a sustained interview rate of 39 subjects per month.

c) *After six months of recruitment (Month 11), the recruitment success will be evaluated and adjustments to the process made as required.* The recruitment process was evaluated after three months of field work. The rate of recruitment of cases was slightly lower than expected and we observed that we were finding more cases than expected in a series of small communities just outside the eligibility boundaries. Therefore, we expanded the recruitment boundaries to include an area 5km outside the original boundaries. The final control recruitment process was evaluated on a monthly basis. No changes were made to the final control recruitment protocol in 2006 based on these reviews.

d) *Interviews will be completed within one month of recruitment. The coordinator will monitor compliance with this target on a monthly basis.* Over the course of the entire field work period, 67% of the cases were interviewed within one month of recruitment. This proportion is adversely affected by the group of cases identified in late 2005 and

early 2006 for whom interviews could not be scheduled until May 2006 (when we obtained final IRB approval). If we exclude men identified prior to May, 2006, the proportion of cases interviewed within one month increases to 75%. For controls, 80% were interviewed within one month of recruitment into the study. For both cases and controls, about 90% of interviews were completed within one month of being assigned to an interviewer, with 50% being completed within 10 days.

e) *All interviews will have been completed by the end Month 19.* As noted above, this target was not met due to personnel and IRB delays. However, all interviews were completed by month 26.

Task #3: PSA testing will be completed on controls within two weeks of collecting the sample. We expect to test around 450 people. (Months 5-19).

This task has been excluded from the project. Our local IRB refused to approve PSA testing on our control subjects (September, 2004). Despite multiple attempts to change the opinion of the IRB, they refused to authorize this component of the project. This was brought to the attention of the HSRRB in 2004. As a result, this PSA testing component was dropped from the protocol. We have retained a question in the interview which obtains a record of any PSA tests which were done in the five years prior to interview.

Task #4: Conduct the calibration sub-study by collecting urine sample on 50 cases and 50 controls (Months 5-21).

This task has been dropped from our SOW primarily due to budgetary limitations. After approval of our protocol, the Canadian dollar rose in value from about $0.75US to $0.92US. This had a major impact on the available Canadian dollar budget; priority was assigned to completing the primary study interviews. When it became clear that the remaining Canadian dollar budget would not support the activities required in the calibration sub-study, a request was made for supplemental funding. This request was denied. The review committee reported that this task was not accepted as a core component of the study. As a result, we had no option but to cease working on this task.

Task #5: Extract DNA from blood samples and complete genotyping of all samples (Months 7-22)

While not listed as an explicit target in the SOW, our biosample collection was very successful. We offered two methods of biosample collection: blood and saliva samples. Overall, biosamples were obtained on 98% of all study participants. The overwhelming majority of subjects (93%) provided a blood sample.

a) *DNA will be extracted from each blood sample within two weeks of collecting the sample.* This target has been met for all samples. Our current laboratory SOP requires the extraction of DNA, and separation of serum, within 2-3 hours of sample collection.

b) *Genotyping will be performed in batches of 400 samples. The first genotyping will be completed by Month 13. Final genotyping will be completed by Month 22.* Our genotyping methods have been modified to reflect improvements and cost reduction in high through-put genotyping. We are currently using an Illumina 96 SNP array, supplemented with taqman assays, to genotype our original list of SNPs. This list of SNPs has been supplemented with additional SNPs addressing other aspects of DNA repair, inflammatory response, and similar systems. A full list of the SNPs being examined can be provided on request. As of the end of 2006, we had completed genotyping on one batch of about 250 subjects. A second batch was genotyped early in 2007. The final genotyping will be done in summer, 2007.

This sub-task has not been fully completed. We have a full genotyping of most of the primary SNPs. However, an issue arose during quality control checks which were conducted in spring, 2008. The genotyping company had supplied results for the first two batches and then provide a cumulative report for the entire study. A comparison of the initial genotype results and the final report indicated major differences in genotype results for several of the SNPs. During summer, 2008, I conducted an independent genotyping (using PCR/RFLP methods) to check the results. This revealed disagreements with the Illumina-based results. I have had several discussions with the genotyping company to resolve this matter. Unfortunately, a range of work and personal issues intervened which led to a delay in obtaining a resolution.

A series of SNPs still remain to be genotyped, all in the NAT1 gene. Following the decision to adopt the Illumina platform technology, it became clear that this gene was not suitable for genotyping using that approach. Rather, a series of PCR/RFLP reactions or taqman assays are required. Given the uncertainty about how to resolve the quality control issues mentioned earlier, I delayed NAT1 genotyping in order to preserve sufficient budget to cover any required re-genotyping.

Reaching a resolution of the remaining genotyping problems is the main reason behind the request for a second no-cost extension. A decision must be reached with the genotyping company about how to handle the quality control issues. One that is resolved, NAT1 genotyping will be performed.

Task #6: Enter all data into the study data base and perform data editing and verification (Months 7-22)

a) *Questionnaire information will be entered into the database as it is collected. Data verification and editing will also take place throughout the study. The finalized data base will be available by Month 22.* Validation of each questionnaire was done by the project staff when the questionnaire was returned to the office. Deficiencies were corrected by contact with the subject as required. All questionnaires have been coded prior to data entry. Questionnaires were entered into the computer through-out the study.

As of March 26, 2009, all questionnaire information have been entered into the computer data base. Data editing has been completed on about 50% of the questionnaire. The second task to be completed during the requested extension is to finalize data editing.

Task #7: Convert food and cooking information into nutrient and heterocyclic amine levels (Months 12-22)

As noted, we have software available to convert food and cooking information. However, this task can not be done until after the final data editing is completed and preliminary data exploration has validated the file.

Task #8: Final analyses and preparation of scientific reports (Months 18-26)

We have completed one secondary analysis and reported the results at the 2008 annual meeting of the American Association for Cancer Research. This abstract reported evidence for a reduced prostate cancer risk associated with higher exposure to sunlight and Vitamin D. Once the data editing is complete, this analysis will be repeated and prepared for publication. At that time, we will be preparing additional papers based on the results of the study. It should be noted that, in epidemiological research, unlike laboratory research, it is uncommon to be able to publish papers based on interim work.

KEY RESEARCH ACCOMPLISHMENTS

Over the course of the past four years, we have completed all primary field work tasks. While some work remains to be completed, these tasks do not require generation of new subject data nor the recruitment of additional subjects. Rather, they reflect the manipulation of the information and biosamples which have been already collected. The following tasks were completed:

- Development of all study protocols and field work methods;
- Hiring of final office and field staff;
- Finalization of subject recruitment and field work methods;
- Development of databases
- Completed interviews on 392 cases and 342 controls
- Obtained biosamples from 386 cases and 333 controls
- All information has been entered into the primary study data base.
- Initial genotyping has been completed on over 100 SNPs

REPORTABLE OUTCOMES

We have completed one secondary analysis and reported the results at the 2008 annual meeting of the American Association for Cancer Research. This abstract reported evidence for a reduced prostate cancer risk associated with higher exposure to sunlight and Vitamin D. Once the data editing is complete, this analysis will be repeated and prepared for publication.

CONCLUSION

Over the past four years, we have successfully conducted a case-control study on over 700 subjects. We were successful in obtaining biosamples from over 96% of the subjects. Final analysis and results are still pending completion of final genotyping and data editing.

- Our protocols were effective in obtaining a very high participation rates. In particular, 92% collection of blood samples is much higher than might have been expected.
- We have obtained some evidence to support the hypothesis that higher intake of Vitamin D may protect against the development of prostate cancer.
- Case recruitment based on review of pathology records can function smoothly.
- Control recruitment has, on the other hand, proved to be more difficult than expected (this is counter to accepted epidemiological teaching). The current legislative environment to enhance privacy has imposed a serious problem in finding suitable control subjects. Control recruitment has essentially been reduced to one strategy: Random Digit Dialing. Standard approaches to random digit dialing are very resource demanding. We had to develop a modified method (in which we use the telephone number of each new case as the base to generate the candidate control telephone numbers). This method has worked well, requiring an average of between 6 and 7 phone numbers to identify a control who agrees to participate.
- Issues in currency conversion can have a profound impact on study viability. Since we developed our initial proposal, the Canadian dollar has risen in value by 20%. Even though I assumed some increase in value when I developed the budget, the increase has been much higher than anticipated and has reduced the available budget by about 10%. This has had a major impact on the resources available for the project. It might be helpful in the future for the funding agency to consider an award process which would include some protection from currency fluctuations.

APPENDIX

FINAL REPORT ON SUBJECT RECRUITMENT(W81XWH-05-1-0148)

N. Birkett

Overview

The PCES is examining the potential impact of increased dietary intake of heterocyclic amines on the risk of developing adenocarcinoma of the prostate. It involves conducting a population-based case-control study with a projected sample size of 400 case and 400 controls. Participants are asked to complete two questionnaires (one self-administered and one administered by a trained interviewer). They are also asked to provide a biosample. The primary biosample was venous blood (collected either at the person's home or at another convenient site) with saliva providing a back-up collection modality. DNA will be extracted from the biosample for genotyping.

This document describes the subject recruitment and interviewing process and results. Initially, a pilot study was conducted from July, 2005 to early 2006. The purpose of this pilot study was to validate study procedures for case recruitment, finalize questionnaires and other study material and to train project staff. Subject identification for the pilot study began with men diagnosed after June 1, 2005 and continued until July 31, 2005.

The control recruitment strategy required three pilot projects to finalize the procedures. Any control subject recruited before May, 2006 was a participant in the pilot phase of the project. Subsequent to that date, the final control recruitment protocol was approved and all control subjects entered the main study. There were a few exceptions related to the training of newly recruited staff; these controls were classified as belonging in the pilot phase even if their recruitment were after May, 2006.

The main study identified men diagnosed with prostate cancer between August 1, 2005 and December 31, 2006 and who were regularly resident within about 10 km of the boundaries of the City of Ottawa. Any candidate subject who failed to meet these criteria was either excluded as ineligible for the main study or enrolled in a sub-study based on a mailed questionnaire (see later).

Control subjects were selected from the general Ottawa population, using a variant on random digit dialing. They were frequency matched on age (decades). Recruitment continued until about May, 2007.

The field work for the main study concluded on June 30, 2007.

All statistics are presented as of March 31, 2009. These are unchanged from the numbers available on June 30, 2007.

CASES

The case identification protocol has been implemented at two hospitals: The Ottawa Hospital and the Queensway Carleton Hospital. The pathology records at the Ottawa Hospital have been reviewed for the months of June, 2005 to December, 2006 inclusive. At the Queensway-Carleton Hospital, the review period was from August, 2005 to December, 2006 inclusive. Subjects identified from reviews conducted in June and July, 2005 at the Ottawa Hospital were eligible only for the pilot study. All other subjects were initially declared as eligible for the main study unless we found evidence that their initial prostate cancer diagnosis had been made prior to August 1, 2005 (e.g. men having post-radiotherapy biopsies) or they lived outside Ottawa (e.g. patients of physicians in Cornwall).

We identified 1,154 pathology reports which met our criteria. About 15% of these reports (178) represented duplicate identification on some subjects. This occurred mainly when a man had a prostate biopsy which was diagnostic for prostate cancer and then, sometime later, had a prostatectomy for treatment (leading to two separate pathological reports on the same subject). A second group of duplicate records related to men having post-radiotherapy biopsies to determine tumour response. These duplicate records were detected prior to any subject contact through the use of an anonymous unique hash-style identifier generated by the Pathology department on each candidate. The second or later identification was excluded from the subject contact protocol. Therefore, the total number of unique candidates for the study is 976. Of these, 84 were identified from pathology reviews which took place in June or July, 2005 and were participants in the pilot study. The remaining 892 subjects were participants in the main study.

Table #1 show the distribution of participation by eligibility status following the pathology review. This eligibility status was based only on the information available to the study pathologist on the pathology reports. The date of diagnosis was recorded as preceding the eligibility date (August 1, 2005) if the pathologist were able to locate any indication in the subject's record to show an earlier diagnosis (e.g. a report that the biopsy was taken to confirm a local recurrence from a tumour diagnosed in 2001).

For the main study, 752 subjects (84%) were deemed eligible for their physician to approached about participation in the study. The two main reasons for being excluded for initial contact by the physician were subjects who had been diagnosed before the eligibility date and subjects who were known to live outside the target area of geographic eligibility. Subjects who lived outside our target area and who were also diagnosed before the eligibility date were classified as ineligible due to their residence rather than their diagnosis date.

Table 1: Status of subjects following anonymous pathology record review

Status	Pilot study	Main study
Eligible candidates	64	752 (84.3%)
Not prostate cancer	0	17 (1.9%)
1st diagnosis precedes eligibility date	15	49 (5.5%)
Too young/old	1	26 (2.9%)
Out of geography	4	48 (5.4%)
TOTAL	84	892

As of August 31, 2007, all mailings from physicians to candidate cases requesting their permission to release their name to the research study had been completed. Letters had been mailed to 49 pilot participants and 707 main study participants. The reason for no mailing being sent to the other 15 pilot subjects and 45 main study subjects are discussed below and shown in Table 2.

For the pilot study, release of name requests were mailed to 49 subjects; 10 eligible candidates were not sent requests to release their name to the study at the request of their physician (eight subjects were reported by their physician to have been diagnosed prior to the eligibility date for the pilot study, one lived outside our interview area and one was excluded for unspecified reasons). A further five candidates were not sent an invitation since were unable to identify a suitable physician to send the letter (their treating physician had recently retired).

Of the 752 eligible candidates for the main study, release requests had been mailed to 707 of them. Thirty-nine (39) subjects were not mailed release requests because the attending physician indicated that this would not be appropriate (nine due to illness or death, 13 due to a diagnosis of prostate cancer before August 1, 2005, one due to language problems, two candidates had moved away from the Ottawa area, one had poor mental status, one had no contact address which could be identified and twelve were excluded for 'non-specified' reasons). Six additional subjects were not mailed a released request since we were unable to identify an appropriate physician to mail the release request.

Table 2: Reasons eligible candidates were not sent an RON request

Status	Pilot study	Main study
MD decision (NOS)	1	11
1st diagnosis precedes eligibility date	8	13
Out of geography	1	2
Too sick/dead	0	9
Poor mental status	0	1
Language problems	0	1
No-address for mailing	5	7
Other (NOS)	0	1
TOTAL	15	45

Table #3 summarizes the status of the 49 subjects in the pilot study and the 707 subjects in the main study to whom name release requests were mailed. In the pilot study, 65% of people asked to release their name agreed to release it. In the main study, 73% of those contacted agreed to release their name to the research study. About 9% of the candidates were abandoned because they failed to respond to their physician after two or three mailings.

Table 3: Response of subjects mailed a request to release their name to the study.

Status	Pilot study	Main study
Returned, release OK	32	513 (73%)
Returned, release denied	9	134 (19%)
Not returned, abandoned	8	60 (9%)
TOTAL	49	707

Table 4 presents the final eligibility status for the people who agreed to have their name released to the study.

In the main study, 79% of the subjects releasing their name to the study were fully eligible. Most of the rest were subjects who were willing to participate but lived outside the interview area (or, in a few cases, had been diagnosed prior to August 1, 2005). This reflected a limitation with the method of recruitment which our IRB has required us to follow: we had no access to the subject's address until after the subject had consented to their name being released. The physicians who mailed the letter to their patients did not have the resources to screen for geographic eligibility. If address information could have been made available to the research office prior to the release of name request being mailed, we could have avoided the recruitment of these ineligible subjects.

In order to avoid undue disappointment for the subjects who had volunteered (often with great enthusiasm) only to discover that they failed to meet the eligibility criteria, we elected to include

these subjects in a sub-study based on a mailed protocol. This involved an expanded version of the self-administered questionnaire combined with a request that the subject provided a saliva sample. This sub-study and associated documents were approved by our IRB and the HSRRB. Details of the sub-study are presented later in this report. Overall, 94 subjects were eligible for the sub-study.

Table 4: Final eligibility status of subjects agreeing to release their name to the study.

Status	Pilot study	Main study
Fully Eligible	23	407 (79%)
Out of area/diagnosis window. Included in sub-study	0	94
Ineligible, pre-August but interviewed	0	4 *
Ineligible, language	0	4
Ineligible, out of area	9	4
Ineligible, too sick	0	0
Ineligible, too old/young	0	0
Ineligible, pre-August (June for Pilot)	0	0
TOTAL	32	513

* Two of the 'pre-August' subjects were interviewed in error. We were not aware of their diagnosis date until the interview had been scheduled.

Table #5 shows the results of the 23 subjects who agreed to take part in the pilot study and the 407 subjects who agreed to take part in the main study interview and who were fully eligible based on the information provided on the Release of Name form. The participation rate amongst subjects agreeing to take part in the study is very high (96%). In the main study, the two ineligible subjects were people who died between their agreement to participate and the interview (there was a lengthy delay in scheduling some interviews due to human resource issues in late 2005). Three subjects were abandoned since we were unable to schedule an interview prior to the end of field work

Table 5: Participation status of eligible subjects agreeing to release their name to the study.

Status	Pilot study	Main study
Participated	22 (96%)	392 (96%) *
Refused	1	10
Ineligible	0	2
Abandoned	0	3
TOTAL	23	407

* Two ineligible subjects were also interviewed (see table 4 footnote)

Table #6 shows the completion status for the three interview components: self administered questionnaire, main interview and biosample. For the interview section of this table, we have included the two ineligible subjects who were still interviewed, making the number of interviewed subjects 394 (rather than 392; the ineligible subjects were not invited to provide a biosample so the number of potential biosamples is only 392). For each component, the table shows both the number of subjects assigned to field work staff and the completion success.

For participants in the main study, questionnaires have been obtained from all but two subjects (99.5% of the participating subjects). One of the two refusals to the main interview was a subject who unfortunately died between completing the self-administered questionnaire and the time of the main interview. The subject's brother hand-delivered the self-completed questionnaire to our office to ensure that we had his brother's information.

Biosample collection has also been very high (98% in the main study) with 94% of biosamples being blood. Only six subjects refused to provide any biosample. This is a very high rate of biosample acquisition, particularly with respect to blood samples. The range of collection options provided to subjects contributed to the high participation rate. Of the subjects providing biosamples, only 8 subjects refused permission to bank their samples for future testing. These samples will be destroyed once the primary genotyping has been completed.

Table 6: Completion status for subjects participating in the interview process

Status	Pilot study	Main study
Self-administered Questionnaire	22	394
• **Completed**	22	392 (99.5%)
• **Refused**	0	2
Main Interview	22	394
• **Completed**	22	392 (99.5%)
• **Refused**	0	2
Biosample	22	392
• **Completed**	22	386 (98%)
• **Refused**	0	6
Biosample type	22	386
• **Blood**	20	363* (94%)
• **Saliva**	2	26*

*Three subjects provided both blood and saliva samples since the blood sample was inadequate for DNA extraction.

SUMMARY

For the time period from June 1, 2005 and August 31, 2007, the study identified 976 candidate case subjects, 84 in the pilot phase and 892 in the main study phase. Attending physicians mailed requests for permission to release their patient's name to the study to 756 subjects (49 in

the pilot phase and 707 in the main study). Permission to release their name to the research study was obtained from 32 pilot phase subjects and 513 main study subjects. Once the name release was obtained, we were able to determine full eligibility: 23 pilot phase subjects and 407 main study subjects met those criteria. All of these subjects (and 2 main study participants who 'slipped through' the eligibility screen during the first months of field work) were scheduled for an interview. Questionnaires were obtained on 414 subjects (22 in the pilot phase and 392 in the main study). Biosamples has been obtained from 408 subjects (22 in the pilot phase and 386 in the main study) with most samples (94%) being blood.

SUB-STUDY

As mentioned above, some cases were recruited to the study before it was determined that they failed to meet the eligibility criteria. If we were aware that a candidate was ineligible before the release of name mailing was sent, they were simply declared to be 'ineligible'. However, we often were unaware that candidates lived outside our target area (since the case identification process precluded any access to an address until after the release of name permission form was returned). In addition, we were sometimes unaware that candidate cases had been diagnosed before the initial eligibility date. Rather than risk antagonising these subjects (who often were very keen to be involved with the project), they were enrolled in a sub-study. This sub-study involved completing a mailed questionnaire (composed of most elements of the main study self-administered questionnaire supplemented with key parts of the main interview questionnaire). In addition, participants were asked to complete a saliva collection kit and return it to the research office. A consent form was signed and returned in a separate envelope.

Ninety-four (94) cases were invited to participate in the sub-study. Of these, 84 lived outside the interview area. The remaining subjects (10) had all been diagnosed prior to the eligibility date of August 1, 2005.

Seventy-four subjects in the sub-study returned a completed questionnaire (79%). All of them returned a saliva sample (100%).

CONTROLS

Development of a successful control identification process involved exploring three different approaches. All of the approaches were based on random digit dialling since there are no sampling frames for the Ontario population to which researchers can still obtain access. The detailed protocols used in the first two approaches required substantial refinement in order to improve recruitment efficiency and feasibility. All controls recruited in the first two approaches were assigned to the pilot study (n=28). With the exception of a small number of subjects recruited for training purposes, all controls recruited by the final method are part of the main study.

The main study recruitment strategy involved placing telephone calls to randomly generated phone numbers within blocks developed from a stem given by the telephone number of each participating case. For example, if a case subject had telephone number 613-555-1234, we would generate a list of random telephone numbers of the form: 613-555-12##. These numbers were activated in groups of five until at least one eligible control subject was recruited. All numbers in the group which generated the eligible control were contacted and could lead to additional controls for the study. Control eligibility was limited to men. Controls were frequency matched to cases using a method in which age ranges were 'open' for recruitment until the age-specific recruitment target had been reached. After initial agreement to participate in the study, control subjects were mailed a form asking if they had prostate cancer. If so, this form was returned to the office and subject was excluded as ineligible. This method followed a protocol approved by our local IRB and the HSSRB.

The main recruitment strategy was effective at recruiting the majority of the control group. However, after 75% of the group had been recruited, it became clear that we were under-recruiting men over age 70. Accordingly, the random digit dialling process was extended to include telephone numbers selected at random from numbers associated with 'independent living' senior's apartments. Finally, we permitted some controls to refer acquaintances to participate in the project.

The full records of the random digit dialling process have not been computerized. Hence, it is not possible to provide a detailed tabulation of the number of telephone calls made, not the outcomes of those calls. Table #7 summarizes the number of blocks and telephone calls which were made to blocks which had been completed prior to August 31, 2006. Note that 2 of the 63 subjects who agreed to participate were actually entered into the pilot study (these were recruited as part of the staff training programme), leaving 61 controls for the main study.

Table 7: Random Digit Dialling blocks and overview of results of calling

Status	Main study
# blocks completed	50
# phone numbers contacted	430
Outcome of contacted numbers	
• **Invalid #**	195 (46%)
• **Abandoned after 13 attempts**	26 (6%)
• **No-one eligible**	97 (23%)
• **Eligible but Refused**	47 (11%)
• **Agreed to participate**	63 (15%)
• **Still pending**	2
# calls required to resolve number	Mean = 2.66
• **1**	272 (64%)
• **2**	48 (11%)
• **3**	32 (7%)
• **4**	11 (3%)
• **5**	12 (3%)
• **6-9**	19 (4%)
• **10+**	34 (8%)
# subjects agreeing to participate	63

Nearly all of the invalid numbers were resolved with one call (95%). Over half (58%) were 'not in service' while 19% were business and 16% were faxes or pagers.

Overall, in blocks completed by August 31, 2006, we had called 430 numbers and made contact with 216 households. From these households, 96 (44%) had no member who was eligible for the study (based on the sex and age of household residents). Of the households with at least one eligible person, 57% had someone who agreed to take part in the interview. We made a mean of about 7 calls to recruit each control subject.

Our monitoring of this process suggests that the call and response patterns remained generally similar through-out the rest of the study. However, as we became more selective in the age groups to which were able to recruit, the number of calls required to recruit a subject increased. We estimate that, overall, we called about 3,500 randomly selected telephone numbers to generate the control group.

We enrolled 419 control subjects into the study: 32 in the pilot and 387 in the main study. These are people who agreed to take part in the process. Some of them subsequently decline to participate or were found to be ineligible. Table 8 shows the source for the controls. Most of the main study controls were obtained from the main RDD method (80%) with the 'enriched' RDD

method (based on senior's apartment buildings) generating most of the remaining controls (16%). Volunteers accounted for only 4% of the control group.

Table 8: Controls: recruitment sources.

Sources	Pilot study	Main study
Main RDD method	0	311
Seniors apart. RDD	0	62
Volunteers	0	14
Pilot methods	32	0
TOTAL	32	387

Table 9 show the eligibility status of the recruited controls. The eligibility proportion (97% in the main study) was very high, largely because subjects who were ineligible were excluded during the initial screening interview. We found that about 2% of our control candidates had previously been diagnosed with prostate cancer.

Table 9: CONTROLS: eligibility status.

Status	Pilot study	Main study
Eligible	30	375 (97%)
Prior prostate cancer	2	8
Poor mental status	0	2
Too sick/died	0	2
TOTAL	32	387

Table 10 presents the participation status for the control subjects who were eligible. For the main study, the participation rate was 92%. Only 6% of control recruits who had agreed to participate subsequently refused the study although we had to abandon attempts to interview nine additional subjects due to protracted difficulty in establishing an interview time.

Table 10: CONTROLS: Participation status.

Status	Pilot study	Main study
Participated	21 *	343 (92%)
Refused	3	23
Abandoned	6	9
TOTAL	30	375

* Two pilot study controls had prostate cancer but were interviewed anyway. They are not included in this table and will not be included in the analysis bur are reported here for completeness.

As seen in Table 11, participation in the various study components was very high. Nearly all subjects completed the two interviews (only one subject refused the self-administered

questionnaire while no subjects refused the main interview). Biosample completion rates were also very high (97%) with 91% of the biosamples being blood.

Table 11: CONTROLS: Completion status for subjects participating in the interview process.

Status	Pilot study	Main study
Self-administered Questionnaire	23	343
• **Completed**	22	342 (99.7%)
• **Refused**	1	1
Main Interview	23	343
• **Completed**	22	343 (100.0%)
• **Refused**	1	0
Biosample	23	343
• **Completed**	21	333 (97%)
• **Refused**	2	10
Biosample type	21	333
• **Blood**	17	303 (91%)
• **Saliva**	4	30

SUMMARY

For the time period from June 1, 2005 and August 31, 2007, the study identified 419 candidate control subjects, 32 in the pilot phase and 387 in the main study phase. In the main study, 12 candidate controls were ineligible due to prior prostate cancers or other issues. A further 23 subjects changed their mind and declined to complete the interview and nine subjects could not be contacted to schedule an interview. Overall, we obtained interviews in 343 control in the main study (92% of eligible subjects). Questionnaires were been returned from all but one control subject. Biosamples were obtained from 354 subjects (21 in the pilot phase and 333 in the main study) with most samples (91%) being blood.